

Search for simple and composite auxin responsive elements in *Arabidopsis thaliana* genome.

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The hormone auxin is a major regulator of plant growth and development. The influence of auxin on gene transcription is primarily mediated through Auxin Response Factors (ARFs). ARFs bind in target promoters to the specific sites called AuxREs (Auxin Response Elements) with the TGTCNN (most frequently TGTCTC) consensus core sequence. While Chip-seq data for most of ARFs are still unavailable, prediction of potential AuxRE is restricted by consensus models that detect too many false positive sites. About half of the *Arabidopsis thaliana* genes have at least one TGTCTC in any orientation within the first 1000 nt of their promoter regions. While single TGTCTC hexamer does not confer auxin inducibility (Ulmasov et al. 1997), this is provided by multimerized (Guilfoyle et al. 1998), or composite AuxREs (Ulmasov et al. 1995). In the composite AuxREs, TGTCNN adjoins or overlaps with coupling elements (Ulmasov et al. 1995; Guilfoyle et al. 1998).

We performed bioinformatical analysis of simple and composite AuxREs distribution in *Arabidopsis thaliana* genome. AuxREs were recognized by three different models: (1) simple TGTCNN consensus, (2) TGTCNN pairs with a certain distance between them and (3) combination of oPWM and SiteGA tools (AuxRE_{P&S}) (Mironova et al., 2014).

To test which model predicts AuxREs associated with auxin response better, we performed meta-analysis of publicly available 23 microarray experiments with auxin treatments (Mironova et al., 2014). First, we created a list of auxin-regulated genes which significantly changed their expression (by more/less than 1,5/0,67-fold, $p < 0,05$) in at least four microarrays. The threshold for the number of microarrays was set by the binomial trial estimate. The resulting list contained 1301 up-regulated and 1262 down-regulated genes. Second, the fractions of the significantly up- or down-regulated genes with an AuxRE variant

in their promoter were compared with that for all the genes tested in the experiment. The statistical significance of the difference between the fractions was estimated by the t-test for arcsine square-root transformed proportions. This analysis showed that all three models predicted AuxREs, which were enriched in auxin responsive genes, but the genes sets differed. For example, a highly associated with auxin response AuxRE_{P&S} were predicted in about of 10% of auxin responsive genes and were associated only with up-regulation. Where several variants of TGTCNN consensus were significantly associated with auxin down-regulation.

Additionally, we performed a context analysis of the flanks in experimentally proven AuxREs and found three distinct types of potential coupling motifs (Y-patch, AuxRE-like, and ABRE-like) (Mironova et al., 2014). The similar bioinformatical analysis of associations in a number of microarray datasets assured us that the composite elements with a specific orientation of AuxRE and the coupling motifs and the certain range of spacer length between them were associated with auxin responsiveness.

The methodology proposed in this work suggested for the cis-regulatory elements annotation in the case the cis-element is associated with a response to physiological and ecological factors.

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